Synopsis of the Thesis entitled

"Toxicity of Electroplating industrial effluent on Freshwater Fish, *Channa gachua* (Ham.)''

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By

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Chapter 1: Introduction

Water is essential and basic need of all living organisms. It consists of many minerals, organic substances and gases at a certain concentration. If the concentration of already present substances, usually not found in water, gets dissolved, water gets polluted. Water pollution refers to have concentration of unwanted chemicals (toxicants) leading to degradation of water quality. Undoubtedly, water pollution is an age old problem, but in this modern age the problem like population explosion, sewage disposal, industrial effluents, radioactive wastes, heavy metals, pesticides, and a number of other resources have polluted our water resources.

Industries are the main source of water pollution. These industries are usually situated on the bank of the rivers and lakes, where they release or dump their waste material that harms the aquatic life, particularly fishes. Polluted water not only poses threat to aquatic species inhabiting them but also to humans who often come in contact with water either directly or indirectly by consuming fish or shell fish.

Industrial discharges containing toxic and hazardous substances including heavy metals harm tremendously to aquatic ecosystem. Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to domestic, industrial and agricultural wastes.

The electroplating industry is one such type of industry. This industry involves deposition of thin protective metallic layer of Zinc (Zn), Chromium (Cr), Nickel (Ni), Copper (Cu), onto a prepared metallic surface, using electrochemical processes. In the plating process the object to be plated is usually used as a cathode in an electrolytic bath. These thin electroplated sheets are used for the purpose of earthing. The effluent generated through this process contain considerable amount of heavy metals like Zinc, Nickel, Chromium and Copper, which are then released in the fresh water bodies. These heavy metals are naturally present in the soil and water bodies as micronutrients at certain concentrations. If the quantity of these metals crosses a particular limit, then they are considered as pollutants. The excessive dumping of these heavy metals from industries severely affects the aquatic life. These metals are non-biodegradable wastes; hence they accumulate in the tissues of aquatic organisms, particularly fishes, which in turn, affect human beings.

Bioassay studies on the acute toxicity of various pollutants have been initiated during the late 19^{th} century. Fishes can be used as test animals for the evaluation of toxicity of

effluents and wastes with the help of toxicity tests. Literature available on the impact of Electroplating industrial effluent as well as heavy metals on different organisms is scanty. The fish, *Channa gachua* is an economically important fish, locally consumed food fish in the region. It is a good indicator of pollution and available throughout the year. The organs selected for the study are Liver, Kidney, Gills, Ovary and Testis who's Histopathology and Biochemical estimations (Protein, glycogen and Minerals) were studied.

Hence the present investigation is planned to study the toxicity of electroplating industrial effluent on freshwater fish, *Channa gachua* (Ham.).

Chapter 2: Physico - chemical parameters of electroplating industrial effluent

The electroplating industry effluent (Sample) was analyzed periodically for physico - chemical characteristics during the course of present study (2010, 2011 and 2012). The physico - chemical data of effluent showed the absence of DO, high BOD, COD, chloride, Sulphate, Phosphate, TSS , TDS, TS levels and low pH values. Moreover, the effluent contained high level of heavy metals like hexavalent Chromium (Cr), Copper (Cu), Nickel (Ni) and Zinc (Zn) whose level was far above the permissible limits as suggested by ISO or WHO. During the course of present study, 17 physico - chemical parameters were considered for analysis of each sample:

Physical parameters:

1. Colour 2. pH 3. TSS. 4. TDS 5. TS

Chemical Parameters:

1. Dissolved Oxygen (DO) 2. BOD (Biological Oxygen Demand) 3. COD (Chemical Oxygen demand) 4. Chloride 5. Hardness 6. Sulphates 7. Phosphates 8. Nitrates 9. Chromium (Cr) 10. Nickel (Ni) 11. Zinc (Zn) 12. Copper (Cu)

Chapter 3: Toxicity tests:

A. Acute Toxicity test:

The acute toxicity tests or "Short-term tests are carried out on different environmental factors that alter the ecological setup and normal aquatic life. Usually the time duration for acute test is 24, 48, 72 and 96 hrs. Acute toxicity tests involve the damage to organism due to reaction mechanism of chemicals.

The test fish, *Channa gachua* were collected from the local market of Aurangabad. The fish is available throughout the year and is easily acclimatized to the laboratory conditions for about 15 days. During the period of acclimatization, fishes were fed on the pieces of earthworm. No food was given to the fishes during experimentation.

The acute toxicity experiments were repeated three times for a concentration with a batch of ten fishes in order to maintain the concentration of the toxicant in the test aquarium. Water was renewed every 24 hours during the short term exposure. Simultaneously a group of control fishes were also kept during experimentation. Only healthy fishes (Length: 12-15 cms; Weight: 50-56 gms) were selected for the experiment.

The percent concentration of test solution has been calculated using the formula; (FAO, 1984)

$$\text{Volume percent} = \frac{\text{Volume of effluent}}{V_{E+}V_{DW}} \times 100$$

V_E = Volume of effluent, V_{DW} = Volume of Dilution water

Toxicity was determined using static bioassay method to estimate LC_{50} values of the respective hours (Sprague, 1973). To evaluate the toxicity, series of different concentrations (21.5%, 21%, 20.5%, 20%, 19.5%, 19%, 18.5%, 18%, 17.5%, 17% and 16.5%) of electroplating industrial effluent were prepared on the basis results based on the pilot tests. At the same time, the acute toxicity of heavy metals like Chromium, Copper, Nickel, and Zinc (in ppm concentrations) were also studied.

The bioassay tests were carried for 24, 48, 72 and 96 hrs. The tests were started early in the morning to observe the behavior of test fishes. The observation on mortality and survival

were noted after every 24, 48, 72 and 96 hours. The fish were considered dead when there was no movement and lack of response to mechanical stimulus.

The LC_{50} values of the electroplating industrial effluent for 24, 48, 72 and 96 hrs were calculated at 20%, 19.5%, 19%, and 18.5% respectively (Table No. 1) by regression analysis method (Finney, 1971). The LC_{50} values for the heavy metal Chromium (Cr) as $Na_2Cr_2O_7$ for 24, 48, 72 and 96 hrs were found to be 180 ppm, 140 ppm, 100 ppm and 60 ppm respectively (Table No. 2). For the heavy metal Copper (Cu) as $CuSO_4$, the LC_{50} values were found to be 180 ppm, 88 ppm, 40 ppm, and 20 ppm for 24, 48 , 72 and 96 hrs respectively (Table No. 3). The acute toxicity of heavy metal Nickel (Ni) as $NiSO_4$ and Zinc (Zn) as $ZnSO_4$ was also studied for 24, 48, 72 and 96 hrs for which the LC_{50} values were estimated at 1200 ppm, 600 ppm, 300 ppm, 150 ppm (Table No. 4) and 630 ppm, 528 ppm, 429 ppm, 330 ppm respectively (Table No. 5).

Table No. 1: LC_{50} values of electroplating industrial effluent for a period of 24, 48, 72 and 96 hrs in freshwater fish, *Channa gachua*

Exposure period in hrs.	LC_{50} Values at % conc.	Regression equation Y = y + b (x – x)	Variance
24	20	Y = 58.2511 X – 70.7813	0.000009
48	19.5	Y = 57.6038 X - 69.3056	0.0000010
72	19	Y = 48.7914 X – 57.3900	0.0000014
96	18.5	Y = 55.4085 X – 65.2093	0.0000010

Table No. 2: LC_{50} values for heavy metal Chromium (Cr) as $Na_2Cr_2O_7$, for a period of 24, 48, 72 and 96 hrs in freshwater fish, *Channa gachua*

Exposure period in hrs.	LC_{50} Values (ppm)	Regression equation Y = y + b (x – x)	Variance
24	180	Y = 35.9232 X – 76.0093	0.000025
48	140	Y = 14.4678 X - 26.0443	0.000152
72	100	Y = 19.8508 X – 34.6738	0.001589
96	60	Y = 11.1578 X – 14.8270	0.002938

Table No. 3: LC_{50} values for heavy metal Copper (Cu) as $CuSO_4$, for a period of 24, 48, 72 and 96 hrs in freshwater fish, *Channa gachua*

Exposure period in hrs.	LC_{50} Values (ppm)	Regression equation Y = y + b (x – x)	Variance
24	180	Y = 9.7799 X – 17.0039	0.000346
48	88	Y = 4.7424 X - 4.1580	0.001484
72	40	Y = 2.0907 X – 1.7683	0.008330
96	20	Y = 3.4451 X – 1.2505	0.166447

Table No. 4: LC_{50} values for heavy metal Nickel (Ni) as $NiSO_4$, for a period of 24, 48, 72 and 96 hrs in freshwater fish, *Channa gachua*

Exposure period in hrs.	LC_{50} Values (ppm)	Regression equation Y = y + b (x – x)	Variance
24	1200	Y = 69.4666 X – 208.8932	6.95×10^{-7}
48	600	Y = 36.9296 X - 97.6150	0.0000249
72	300	Y = 17.7722 X – 38.9801	0.000104
96	150	Y = 7.3779 X – 11.0345	0.000622

Table No. 5: LC_{50} values for heavy metal Zinc (Zn) as $ZnSO_4$, for a period of 24, 48, 72 and 96 hrs in freshwater fish, *Channa gachua*

Exposure period in hrs.	LC_{50} Values (ppm)	Regression equation Y = y + b (x – x)	Variance
24	**630**	**Y = 8.9540 X – 20.0639**	**0.000403**
48	**528**	**Y = 9.5268 X - 20.9329**	**0.000365**
72	**429**	**Y = 3.8744 X – 5.1841**	**0.002215**
96	**330**	**Y = 12.3394 X – 26.0704**	**0.000213**

B. Chronic Toxicity Tests:

The Chronic toxicity tests deals with the chronic toxicity or long term toxicity of electroplating industrial effluent, studied by exposing the test fish to two sublethal concentrations. The sublethal concentrations were selected on the basis of the results of acute toxicity values. The LC_{50} values for 96 hrs were estimated at 18.5% of the electroplating industrial effluent. The sub-lethal concentrations for chronic tests were selected at 3.70% ($1/5^{th}$ of 96 hrs) and 1.85% ($1/10^{th}$ of 96 hrs) for a period of 30 days. Similarly, the fishes were also exposed to the two sublethal concentrations of the heavy metals. For the heavy metal Chromium, the two sublethal concentrations are 12 ppm ($1/5^{th}$ of 96 hrs) and 6 ppm ($1/10^{th}$ of 96 hrs); for the heavy metal Copper, the two sublethal concentrations are 4 ppm ($1/5^{th}$ of 96 hrs) and 2 ppm ($1/10^{th}$ of 96 hrs). The fishes were also exposed to the heavy metals Nickel and Zinc, at two sublethal concentrations i.e., 30 ppm ($1/5^{th}$ of 96 hrs) and 15 ppm ($1/10^{th}$ of 96 hrs) for nickel and 66 ppm ($1/5^{th}$ of 96 hrs) and 33 ppm ($1/10^{th}$ of 96 hrs), for zinc, respectively.

Effects of the two sublethal concentrations of the effluent as well as heavy metals have been studied considering the aspects like survival, feeding, growth and histology of the tissues like Liver, Kidney, Gills, Ovary and Testis.

Survival: The survival of the experimental fish exposed to electroplating industrial effluent and the heavy metals is overall 100%.

Feeding: The food consumed by *Channa gachua* was recorded weekly. Percent increase or decrease in the intake of food during the exposure periods have been recorded which revealed

that the treated fish has decreased the rate of food intake. In case of control group, slightly increased intake of food was recorded.

Growth: Growth of *Channa gachua* has also been studied in both control as well as chronically exposed fishes. Weekly observations were made to study the growth in relation with body weight. The results on the growth revealed that the growth of control fish increased progressively. The pattern of growth in treated fish showed slight decrease during the 1st, 2nd, 3rd and 4th weeks.

Chapter 4: Histopathological study:

Histopathological research has made, directly or indirectly a great contribution in better understanding of physiology of fish. It is well known that histological approach is the most valuable tool for accessing the action of toxicant at tissue level for providing data concerning tissue damage and manifestation of structural and functional changes in tissues and organs.

In the present investigation, the effects of electroplating industrial effluent as well as heavy metals like Chromium, Copper, Nickel and Zinc, on the experimental fish *Channa gachua* at acute and chronic exposures were recorded. Fishes were divided in two groups, as control and experimental.

At the end of exposure periods the survived fish were sacrificed, dissected and the organs like Gills, liver, Kidney, ovary and testis were preserved in the bouins' fixative. The tissues were processed and embedded in paraffin wax. Sections at 4-6µ thickness were cut and stained in Harrys' Haematoxylin – Eosin (H/E) as suggested by Bancroft and Stevens (1977) and Bancroft and Cook (1984). After staining the slides were mounted in DPX and observed under the light microscope and histological details and subsequent photomicrographs were taken.

Normal histology of tissue:

1. **Gills:** *Channa gachua* possesses four pairs of gills situated at branchial chamber, as in other teleosts. Each gill arch has a double row of elongated, laterally projecting structures, the primary gill filaments. On the upper and lower surface, leaf-like projections at right angles to its axis is the secondary gill lamellae. The whole area of secondary gill lamellae is occupied by a continuous row of pillar cells.
2. **Liver:** The liver is a digestive gland composed of hepatic cells (Hepatocytes). Serous membrane covers the surface of liver and some connective tissues from this capsule

extends inward into parenchyma. The portal vein entering the liver carries venous blood from stomach and intestine. It branches off and eventually divides into relatively wide blood capillaries known as sinusoids. The hepatic cells are generally round or polygonal and contain a prominent nucleus. Bile is secreted by the hepatic cells and poured into duodenum through bile canaliculi. Metabolism of food and storage is an important function of the liver. The teleostean liver is also concerned with excretion and detoxification of harmful substances without having direct contact with them.

3. **Kidney:** The fish kidney comprises of head and trunk kidney, the former in most cases refers to pronephric whereas the later represents the functional kidney of fish. A large number of uriniferous tubules are present in the kidney that drains the excretory waste material to the exterior. The uriniferous tubules have components like glomerulus and tubules, the former made up of network of capillaries lodged into bowmans capsule and filter urine retaining the large molecules at side of the bowmans' capsule. The collecting tubules – the proximal and distal convoluted tubules, hematopoietic tissue are also seen in the connective tissue of the kidney.
4. **Ovary:** Under control conditions, the ovary shows a number of Oocytes showing various stages of maturation. Immature oocytes are small in size with large nuclei and several nucleoli scattered in the nucleus. In some oocytes nucleoli are arranged on the periphery of the nucleus. Fatty yolk globule formation starts from the periphery of the oocytes.
5. **Testis:** In the histological study, Testis showed seminiferous tubules, embedded in the connective tissue. The seminiferous tubules contain primary spermatocytes, secondary spermatocytes, spermatids and sperms are containing a large no of sperms. In between the seminiferous tubules, interstitial cells are present.

Histopathological Changes:

On acute and chronic exposure to electroplating industrial effluent and heavy metals like Chromium, Copper, Nickel and Zinc, the experimental fishes showed the following degenerative changes in the different organs like gills, liver, kidney ovary and testis

1. **Gills:** The gills exposed to lethal and sublethal concentration of electroplating industrial effluent and heavy metals (Chromium, Copper, Nickel and Zinc) showed degeneration and breaking of cellular epithelium and vacuolation of the secondary gill lamellae. Destruction of blood cells, blood capillaries and nuclei, Fusion of secondary lamellae, Curling of primary and secondary lamellae were the prominent features observed in the gills.

2. **Liver:** On acute and chronic exposure to electroplating industrial effluent and heavy metals like Chromium, Copper, Nickel and Zinc, the experimental fish showed degenerative changes in the hepatic tissue like necrosis in hepatocytes, shrinkage and destruction of central vein, rupture of sinusoids, disintegration of islets of Langerhans, degeneration in bile canaliculi, vacuolation and necrosis in hepatic tissue.
3. **Kidney:** Kidney showed degeneration of glomerulus, shrinkage of renal tubules, hemorrhage and necrosis in hematopoietic tissue, necrosis and loosening of connective tissue, degeneration of blood vessels, vacuolation, cellular atrophy, and reduction in lumen of proximal tubules, and pyknotic nuclei. Appreance of eosinophilic granules in a large number of epithelial cells bordering the tubules were also observed on acute and chronic exposure to electroplating industrial effluent as well as the heavy metals like Chromium, Copper, Nickel and Zinc.
4. **Ovary:** In the experimental conditions, prominent histopathological changes are observed such as degeneration of oocytes, majority of oocytes are immature; they tend to lose shape and show drastic reduction in size. Atreic occytes increase in number, rupture of ovigereous lamellae, degeneration of connective tissue on exposure to 24, 48, 72 and 96 hrs (acute) and 30 days (chronic) exposure to electroplating industrial effluent as well as heavy metals like Chromium, Copper, Nickel and Zinc.
5. **Testis:** Testis showed severe degenerative and necrotic changes in the cellular elements of the seminiferous tubules with focal areas of necrosis and degeneration in the walls of seminiferous tubules. Seminiferous tubules appeared with lesser number of sperms indicating lack of active spermatogenesis. Also vacuole formation in seminiferous tubules, degeneration of primary spermatocytes, Degeneration of interstitial cells, disintegration of blood vessels, and necrosis in connective tissue were the prominent features.

Chapter 5: Biochemical studies

This chapter includes 3 aspects: 1. Protein estimation.

2. Glycogen estimation

3. Mineral estimation

1. Protein estimation: Proteins are the basic molecules to any living system. In cells they function as enzymes, structural material, lubricants and carrier molecules. A significant decrease was found in the protein levels in all vital organs selected for the study ie., Gills, Liver, Kidney, Ovary and Testis after exposure to the electroplating industrial effluent a

well as on exposure to different heavy metals like Chromium, Copper, Nickel and Zinc. The depletion of protein content during all exposure periods suggests as increased proteolysis and possible utilization of the products of their degradation for metabolic purposes.

2. Glycogen estimation: Carbohydrates play a structural role as well as they act as a reservoir of chemical energy to be increased or decreased according to the organisms need. Glycogen in the tissues is also considered as an immediate source of energy to adapt to the environmental conditions. A highly significant decrease was found in all the vital organs, especially liver and kidney, after exposure to the electroplating industrial effluent and on exposure to heavy metals (Chromium, Copper, Nickel and Zinc). Decrease in the glycogen levels suggests the possibility of glucogenolysis which in turn produce energy to cope up the adverse stress conditions.

3. Mineral estimation: **Minerals estimated: 1. Sodium (Na)**

2. Potassium (K)

3. Calcium (Ca)

a.) Sodium (Na): It is one of the chief regulators of osmotic pressure of body fluids. On exposure to electroplating industrial effluent and also on exposure to heavy metals like Chromium, Copper, Nickel and Zinc, it was observed that a significant decrease of sodium occurs in the liver, gills, ovary and testis whereas a non-significant increase of sodium ions was observed in the kidney.

b.) Potassium (K): It is the major intracellular cation involved in several physiological functions i.e., nerve and muscle function, acid - base function, and osmotic pressure. In this study, a decrease in the potassium content was found in all the organs ie., gills, liver, kidney and gonads which might be due to disturbed potassium regulation.

c.) Calcium (Ca): Calcium is the major ions required for the density of bones. Decrease of calcium level in all the vital organs (Liver, gills, Ovary, Testis) whereas a non-significant increase in the kidney was observed during the acute and chronic exposure periods to the pollutants. It might be due to accumulation of calcium from the entire vital organs- (Gills, liver, ovary, and testis) to the organ kidney.

Chapter 6: Conclusion

The contamination of heavy metals is a serious threat to the aquatic organisms because of their toxicity, long persistence, bioaccumulation and biomagnification in the food chain. Toxicity of heavy metals is time dependant and also on the nature of heavy metal. The present study reveals that electroplating industrial effluent containing heavy metals has a tangible effect on the fresh water fish, *Channa gachua*. Similar results were obtained on exposure of the test fish to the heavy metals- chromium, copper, nickel and zinc. Thus it is suggested that the electroplating industrial effluent should be pretreated to remove the heavy metals and also to neutralize the pH before releasing it in the natural water bodies, thus to conserve the aquatic ecosystem.

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